Transitioning novel peptide hits into lead compounds

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Developing novel peptide hits into lead compounds can be challenging and requires a modified approach compared to small molecules. When screening for hits on difficult drug targets such as orphan G-protein coupled receptors (GPCRs) or ion channels it is often necessary to go outside of the Lipinski rule of five. These compounds are in libraries which could include natural products, peptides, fragments etc. Finding the hit is the first step, and this then may be used as a tool to rescreen small molecules libraries with better confidence. But often this new hit needs to be investigated as potential lead material to progress the project from a potentially stalled situation. Unlike synthetic compound libraries, natural product hits need to be identified and then characterised as the actual molecule is often unknown. Peptide libraries come from a range of sources and they all have limitations and benefits. Venom peptides are often inherently stable due to cysteine knots whereas other peptides may not be. This article will take on the challenges of the hit-to-lead journey with these non-standard hits.

Towards the end of the 20th century there was a strong drive to uncover critical parameters that would produce more successful drug candidates. This lead to the gold standard of developing Lipinski rule of five compliant compounds, first published by Pfizer in 1997 (1). This was driven by the large attrition rate of compounds in the clinic which was very costly in time and money. However, rule of five compliant compounds have not managed to address all drug targets and a new approach is needed. With so called difficult targets such as orphan GPCRs, ion channels and non-receptor targets it is often necessary to go beyond the Lipinski rule of five (2). Biologicals such as antibodies and peptides have a greater opportunity for selectivity and thus reduced side effect profile due to greater number of contact points with the target protein (3). It is logical to investigate a peptide inhibitor of a protein-protein interaction as they contain the same functional group chemistry as the system to be perturbed. Peptide and antibody libraries can be screened using the same platforms used for small molecules to identify hits as starting points for drug discovery projects.

When novel peptide hits are being investigated, the same rules of potency and selectivity need to be assessed as for any other compound. A hit from a natural compound library could a contain a number of entities. These need to be further separated out and single hits need to be confirmed (dose response) and identified utilising mass spectrometry (4). This information can be plugged into structure activity relationships (SAR) tables to identify regions where variability is affecting potency and or selectivity. There is a benefit of venom peptides over some other libraries which comes from closely related species often having single amino acid changes that can be used in SAR thus avoiding the need for systematic mutation. However, peptide scanning can still be a useful technique to support the drug discovery process as source material for structure-activity relationship studies (5). SAR is considered more challenging for peptides as they are complex molecules but the functional unit, the amino acid, is also larger and thus the same principles can be applied. Also, peptide leads can be synthesised easily, compared to small molecule natural products where the synthesis path can be much more difficult to produce. Peptide SAR can be used to improve stability and pharmacokinetics (PK) as for other molecules. Peptide hits can also be further developed into promising lead material through directed evolution. This has been used to great effect for difficult

targets such as NaV1.7 (6). Directed evolution is achieved through cloning the peptide gene into an expression vector, amplifying with error prone mechanisms and screening the resulting libraries. This technique has many benefits over SAR as the random mutations can identify sequences with beneficial pharmacological properties that could not have been predicted through SAR.

When transitioning from initial hits to leads it is important to have multiple chemical series, to mitigate circumstances when a problem is associated with the lead series rather than the target. Convergent evolution has provided a serendipitous collection of unrelated compounds that hit the same targets. Venom systems of snakes, spiders and anemones are completely unrelated as they share no common venomous ancestor, yet all three groups contain peptide blockers of ASIC channels with differing selectivity profiles. Figure 1 shows no homology between the peptide sequences however they all have the potential to be developed as leads for ASIC channel targets. Mambalgins demonstrate the power of natural variation to understand SAR as mambalgin 1 and 2 are both 57 amino acids, three finger peptides with four disulphide bonds that only differ by a single amino acid. Mambalgin-2 differs from Mambalgin-1 by a single amino acid at position 4 (tyrosine in mambalgin-1 and phenylalanine in mambalgin-2) which confer different pharmacological properties (7).

Some earlier venom derived drugs have come from pathology or venom biology rather than classic drug discovery. Eptifibatide (Integrilin) which is derived from a peptide produced in the venom of a rattlesnake, utilised in antithrombotic management is an example of this (8). Echistatin is a potent platelet aggregation inhibitor discovered in a viper (9). Ziconotide (Prialt®) was developed as a pain therapeutic from a cone snail venom peptide ω-conotoxin MVIIA peptide approved in 2004 (10) and Exenatide (Byetta®) a peptide called exendin-4 from the venom of the Gila monster lizard in the treatment of diabetes was brought to market in 2005 (11).

Screening of venom libraries for drug discovery have led to developments currently in clinical trials which include Chlorotoxin, a peptide from a scorpion that blocks chloride channels and has been used to image tumours (12). There are also new developments derived from spider venom, for example in the treatment of epilepsy. Inhibitors of a key antiepieptic drug target, the human ether à go-go voltage-gated potassium channel 1 (hEAG1) have been developed from the spider peptides Aa1a and Ap1a which target both the activation and inactivation gating of the channel (13). Analgesic efficacy has also been targeted with a peptide leads Ca2a targeting NaV1.7 which is derived from spider venom (14).

Most drugs are developed with the intention of oral bioavailability, due to greater patient compliance, so this is considered early in the drug discovery process (15). The general opinion is that peptides are not stable in the gastrointestinal tract, however natural peptides have been shown to have oral activity in mammals (16) and insects (17). Improved oral availability can be left to the development phase as there are many technologies such as formulation with excipients or conjugation with polymers such as polyethylene glycols which have been shown to solve such problems (18). Beyond lead development peptides can be further developed with chemical modifications such as cyclisation, non-natural amino acids and stapling (3). All these modifications have been shown by multiple authors to improve critical parameters such as absorption, plasma half life and metabolism which all contribute to overall bioavailability.

The Lipinski compliant compounds have delivered many excellent drugs however numerous key targets remain unprosecuted. To address these difficult drug targets will require a different

approach and novel chemical entities including biologicals which are already showing promise in this area. The hit to lead process is comparable between small molecules and biologicals and thus many of the technologies can be employed in development of novel therapeutics for the future.

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